Acinetobacter baumannii virulence assay in Caenorhabditis elegans

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Overview of Organisms and Bacteria
Acinetobacter baumannii (Ab):
• Is an opportunistic pathogen
• Has a bimodal DNA Damage response
• Quickly acquires antibiotic resistance

Caenorhabditis elegans:
• Is a model organism
• Only has an innate immune system

Acinetobacter baumannii ΔrecA:
• Is the second strain of Ab used
• Produces more biofilm than Ab wt
• Is more mutagenic

Caenorhabditis elegans Δdaf-7:
• Has a loss of function in a neuronal signaling pathway
• Is perpetually in the Dauer (Starved) state of the C. elegans life cycle
• Has lower feeding rates, a smaller size, and a longer lifespan

Methods
C. elegans are cultured on an agar plate containing E. coli

A day prior to the assay, some C. elegans are transferred to a new plate containing E. coli

The C. elegans eat all of the E. coli and are transferred to the assay

Agar Plate (not to scale)

The C. elegans are mixed with methylene blue and a bacteria strain in a 96 well plate

The mixture is allowed to incubate at room temperature for 3 hours

The mixture is pipetted onto an agar plate where the liquid dries off

3 hours

Well (not to scale)

Agar Plate (not to scale)

C. Elegans Pictures
A: Pictures of C. elegans after mixing with E. coli liquid cultures in the assay, 50% survival rate, taken at 40x magnification
B: Pictures of C. elegans after mixing with A. baumannii ΔrecA liquid cultures in the assay, 35% survival rate, taken at 40x
C: Pictures of C. elegans after being in A. baumannii ΔrecA liquid cultures in the assay, 0% survival rate, taken at 40x

Methylene Blue Tests
A: Results of the assay when performed with and without blue dye mixed with E. coli liquid cultures
B: Results of the assay when performed with and without blue dye mixed with A. baumannii ΔrecA liquid cultures

Note for A. baumannii ΔrecA: the p-value was found to be 0.572, which is higher than 0.05 so there is no statistical difference between cultures with and without methylene blue dye

Conclusion
• The assay worked and showed interesting interactions between strains of C. elegans and strains of A. baumannii
• C. elegans fed with E. coli had a higher survival rate than when fed with either strain of A. baumannii
• C. elegans Δdaf-7 seems to not be affected by A. baumannii ΔrecA, and this is thought to be because the immune system of the worm is unable to respond to the bacterial presence
• The C. elegans ΔrecA strain is very affected by A. baumannii ΔrecA, and this is thought to be due to a change in the surface envelope of A. baumannii ΔrecA that interacts differently with the C. elegans immune system leading to worm death
• The observed C. elegans death in an incredibly short time frame, three hours, which corresponds to the initial stages of bacterial infection in a multicellular host. This will allow us to determine genes in A. baumannii important for the initial steps of pathogenesis

Future Directions
• Testing of more A. baumannii and C. elegans strains
• Determining the timeframe of infection